

University of Groningen

When synthetic cells and ABC-transporters meet

Sikkema, Hendrik

DOI:
[10.33612/diss.136492038](https://doi.org/10.33612/diss.136492038)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Sikkema, H. (2020). *When synthetic cells and ABC-transporters meet*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.136492038>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

When ABC-transporters and synthetic cells meet

Hendrik R. Sikkema



**university of
groningen**

**faculty of science
and engineering**



The work described in this thesis was performed in the Membrane Enzymology group of the Groningen Biomolecular Sciences and Biotechnology Institute (GBB) at the University of Groningen, the Netherlands. The work was funded by the Netherlands Organization for Scientific Research (NWO) and by the European Research Council (ERC).

Cover design: Hendrik R. Sikkema, courtesy illustration synthetic cell to Bert Poolman.

Cover image: The construction of a synthetic cell, with OpuA represented in green and the enzymes arginine deiminase, ornithine transcarbamoylase, carbamate kinase, and the arginine/ornithine antiporter of the ADI pathway represented in orange

Printed by: Ipskamp Drukkers BV, Enschede, The Netherlands



rijksuniversiteit
 groningen

When ABC-transporters and synthetic cells meet

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

vrijdag 13 november 2020 om 12.45 uur

door

Hendrik Reinier Sikkema

geboren op 1 november 1991
te Smilde

Promotores

Prof. dr. B. Poolman

Prof. dr. D.J. Slotboom

Copromotor

Dr. C. Batista Paulino

Beoordelingscommissie

Prof. dr. D.C. Rees

Prof. dr. G. van den Bogaart

Prof. dr. P.C.A. van der Wel

*I dedicate this dissertation to
Valentina, my family and my close friends
who provided me with the inspiration and energy to write it.*

*Enjoy the little things,
for one day you may look back and realize they were the big things.*

Robert Brault

Contents

Scope of this thesis	xiii
1 Cell Fuelling and Metabolic Energy Conservation in Synthetic Cells	1
1.1 Introduction	3
1.1.1 Synthetic life	3
1.1.2 Coupling of exergonic and endergonic reactions and measure of energy status	4
1.2 Cell fueling systems	5
1.2.1 Arginine breakdown pathway	6
1.2.2 Decarboxylation pathways	7
1.2.3 Artificial photosynthetic cells	9
1.2.4 Molecular rheostat	9
1.3 Compartmentalization and vesicle systems	12
1.3.1 Building blocks for membranes	12
1.3.2 Membrane crowding	12
1.3.3 Vesicle systems	13
1.3.4 A metabolic network for energy and physicochemical homeostasis	14
1.3.5 Sensors to measure the energy and physicochemical status of cells	14
1.4 How much ATP does a synthetic cell need?	15
1.4.1 Synthesis of proteins	16
1.4.2 Synthesis of information carriers	16
1.4.3 Lipid synthesis for compartmentalization	17
1.4.4 Membrane transport for osmotic, ionic and pH control	18
1.4.5 Maintenance energy	18
1.4.6 Quantification of ATP demand of minimal synthetic cell	19
1.5 Outlook and perspectives	20
1.6 Open questions	21
1.7 Acknowledgements	21
References	21
2 A synthetic metabolic network for physicochemical homeostasis	31
2.1 Introduction	33
2.2 Results	34
2.2.1 A system for sustained production of ATP	34
2.2.2 Engineering of the metabolic network for ATP	34
2.2.3 Arginine breakdown and control of futile hydrolysis and pH.	38
2.2.4 Load on the metabolic network	43
2.2.5 Physicochemical homeostasis	45

2.3	Materials and methods	47
2.3.1	Materials	47
2.3.2	Construction of expression strains	48
2.3.3	Expression of genes	49
2.3.4	Preparation of cell lysates and membrane vesicles	50
2.3.5	Purification of ArcA, ArcB, and ArcC1	50
2.3.6	Purification of PercevalHR	51
2.3.7	Enzymatic assays for ArcA and ArcB	51
2.3.8	Enzymatic assays for ArcC1	51
2.3.9	Purification of ArcD2 and OpuA	53
2.3.10	Light scattering for oligomeric state determination	53
2.3.11	Co-reconstitution of ArcD2 and OpuA	53
2.3.12	Encapsulation of the arginine breakdown pathway	54
2.3.13	Cryo-EM analysis of vesicles	56
2.3.14	Transport assays	56
2.3.15	Internal ATP:ADP ratio measurements with PercevalHR	57
2.3.16	Internal pH measurements with pyranine	57
2.3.17	External pH measurements with pyranine	58
2.3.18	Amino acid and ammonia analysis	59
2.3.19	Membrane permeability with stopped-flow fluorescence	59
2.4	Acknowledgements	62
2.5	Contributions	63
	References	63
3	Gating by ionic strength and safety check by cyclic-di-AMP in OpuA	69
3.1	Introduction	71
3.2	Results	71
3.2.1	Functional properties of OpuA	71
3.2.2	Architecture of OpuA	72
3.2.3	Substrate loading of OpuA	79
3.2.4	Regulation of OpuA by ionic strength	82
3.2.5	Regulation of OpuA by cyclic-di-AMP	83
3.2.6	Transport cycle of OpuA and conclusions	90
3.3	Materials and methods	90
3.3.1	Materials	90
3.3.2	Expression of OpuA and preparation of membrane vesicles	91
3.3.3	Purification of OpuA	92
3.3.4	Labeling of OpuA and accessibility of scaffold domain	92
3.3.5	Purification of MSP1D1	92
3.3.6	Reconstitution of OpuA in MSP1D1 nanodiscs	93
3.3.7	ATPase activity assays	93
3.3.8	Cryo-EM sample preparation and data acquisition	94
3.3.9	Image processing	94
3.3.10	Model building	95
3.3.11	Labeling of OpuA for single-molecule FRET	96
3.3.12	Single-molecule FRET	96

3.3.13	Co-reconstitution of ArcD2 and OpuA in liposomes	97
3.3.14	Encapsulation of the arginine breakdown pathway	97
3.3.15	<i>In vitro</i> transport assays.	98
3.3.16	<i>In vivo</i> transport assays	98
3.4	Data availability	98
3.5	Acknowledgments	99
3.6	Author contributions	99
	References	99
4	Heterodimer formation of the homodimeric ABC transporter OpuA	105
4.1	Introduction	107
4.2	Results	109
4.2.1	Verification of activity with different affinity tags	109
4.2.2	Heterodimer formation	109
4.2.3	Homologous recombination	111
4.2.4	Optimization of induction	111
4.2.5	TwinStrepII-tag.	113
4.2.6	Optimization of reconstitution	114
4.2.7	Purification of the OpuA-HSS heterodimer	114
4.3	Discussion	115
4.3.1	Spatial separation.	115
4.3.2	Multimerization interface.	117
4.3.3	Expression conditions	117
4.4	Materials and methods	117
4.4.1	Materials.	117
4.4.2	Construction of strains and growth conditions.	117
4.4.3	Expression of <i>opuABC</i> genes	119
4.4.4	Optimization of the induction conditions	119
4.4.5	Isolation and preparation of membrane vesicles	120
4.4.6	Purification of OpuA	120
4.4.7	SDS-PAGE and Western blotting analysis.	122
4.4.8	ATPase activity assay.	122
4.5	Acknowledgements.	122
	References	122
5	A versatile <i>in silico</i> approach to find residues for FRET/EPR pairs	127
5.1	Introduction	129
5.2	Results	130
5.2.1	The ABC-transporter OpuA	130
5.2.2	<i>In silico</i> distance mapping	130
5.2.3	Filtering of the results	131
5.2.4	Mutations in OpuAC, the substrate-binding domain of OpuA	134
5.2.5	Mutations in the full-length transporter OpuA	135

5.3	Discussion	135
5.4	Methods	137
5.4.1	Construction of expression strains	137
5.4.2	Expression of genes	137
5.4.3	Isolation and purification of OpuAC	137
5.4.4	Labeling of OpuAC for single-molecule FRET	137
5.4.5	Isolation and purification of OpuA	138
5.4.6	Reconstitution of OpuA in MSP1D1 nanodiscs	138
5.4.7	Labeling of OpuA for ATPase assay	139
5.4.8	ATPase activity assays	139
5.4.9	Single-molecule FRET	139
5.4.10	Python code for automatization	140
5.5	Acknowledgements	143
5.6	Contributions.	143
	References	143
6	Perspectives	147
6.1	Introduction	149
6.2	Synthetic cell.	149
6.2.1	Energy and other requirements	149
6.2.2	Pysicochemical- and other constraints	149
6.2.3	Building the synthetic cell and startup	150
6.2.4	Bridging the gap	150
6.2.5	Bio-orthogonal expansion	150
6.2.6	Cyclic-di-AMP and osmoregulation.	151
6.3	OpuA	151
6.3.1	Single-molecule FRET	152
6.3.2	Cryo-Electron microscopy	153
6.4	A brief view on the future of the field.	154
	References	155
7	Appendices	161
7.1	Scientific summary.	162
7.2	Wetenschappelijke samenvatting	164
7.3	Popular summary.	166
7.4	Samenvatting voor leken	168
7.5	Резюме для неспециалистов.	170
7.6	Acknowledgements.	172

Scope of this thesis

This thesis covers multiple aspects of (synthetic) biochemistry. Starting from a global point of view and then going deeper into signalling on the single protein level. It provides insight into the state-of-the-art (synthetic) biochemistry and showcases the importance by zooming in on details and zooming out to see the broader context.

The first chapter provides a detailed analysis of important design principles of synthetic cells. The focus lies on the energy balance of the cell. We discuss several systems to (re)generate metabolic energy and give an estimation on how much energy in terms of ATP is needed to maintain a (synthetic) cell.

In chapter 2 we use one of the systems that was presented in chapter 1 in the context of a cell-like environment. We have developed a system in liposomes that is able to use external arginine as a fuel to regenerate ATP on the inside of the vesicle. We show basic physicochemical homeostasis and use the ATP that is produced to fuel one of the key proteins in osmoregulation, the ABC transporter OpuA. In case of an osmotic upshift, OpuA is activated by ionic strength and is able to import the compatible solute glycine betaine against large concentration gradients, which is powered by ATP.

In chapter 3 we focus on this protein. With use of single particle cryo-electron microscopy we have obtained a number of structures of OpuA in multiple conformations that help in understanding the transport mechanism. We also show that OpuA is regulated by the second messenger cyclic-di-AMP, which acts as an emergency brake.

Chapters 4 and 5 focus on methodological advances towards single-molecule FRET studies on OpuA. First we demonstrate how we turned OpuA from a homodimeric into a heterodimeric protein complex (chapter 4). When homodimeric proteins are labeled with probes for *e.g.* smFRET or DEER spectroscopy, any mutation introduced in one protomer also arises in the subunit of the dimer. Transforming the protein into an apparent heterodimer, by tagging the two identical subunits differently, circumvents this problem.

Then, in chapter 5 we introduce an *in silico* approach to find new positions for labeling that can be used for smFRET or DEER spectroscopy. The approach uses two or more crystal structures as input and then systematically assesses all possible residue pairs and filters out positions with suitable accessibility and spacing.

The final chapter (chapter 6) places the work presented in this thesis into perspective and provides a view on the possible future of the research.

